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Analysis of Environmental Releases and Occupational Exposure in Support of Proposed TSCA 5(h)(4) Exemption

I. Introduction

In the 1986 EPA Statement of Policy (51 FR 23303), the Agency stated its intent to pursue a TSCA Section 5(h)(4) exemption for closed-system uses of genetically engineered microorganisms (GEM's). Eligibility for the 5(h)(4) exemption is expected to be based on the identity of the recipient microorganism, limitations on the types of genetic material which may be introduced to the recipient organism, and containment criteria. The criterion for this type of exemption is the finding of "no unreasonable risk." In order to make such a risk determination, EPA is analyzing the potential hazards and exposures associated with certain GEM's. This report quantifies the potential releases to the environment and occupational exposures from a closed system fermentation process and will support the overall risk assessment.

II. Approach

Potential worker exposures and routine releases to the environment from a large-scale, conventional fermentation process have been assessed based on available information including eight Premanufacture Notices submitted to EPA under Section 5 of TSCA and published information collected for non-engineered microorganisms. Reasonable worst case assumptions are used and wherever possible,

ranges and/or typical values are presented for comparison. Operating conditions, exposures, releases, and effectiveness of controls will vary for each operation. This assessment may not adequately represent each individual fermentation process but is expected to be representative of a large number of such operations. Additionally, it provides a means of comparing the potential environmental releases for different levels of containment.

This assessment is based on the limited information available to date and will be the basis for developing a generic scenario to assist the Chemical Engineering Branch in the assessment of biotechnology submissions under TSCA Section 5. As new information becomes available, it will be incorporated into the generic scenario.

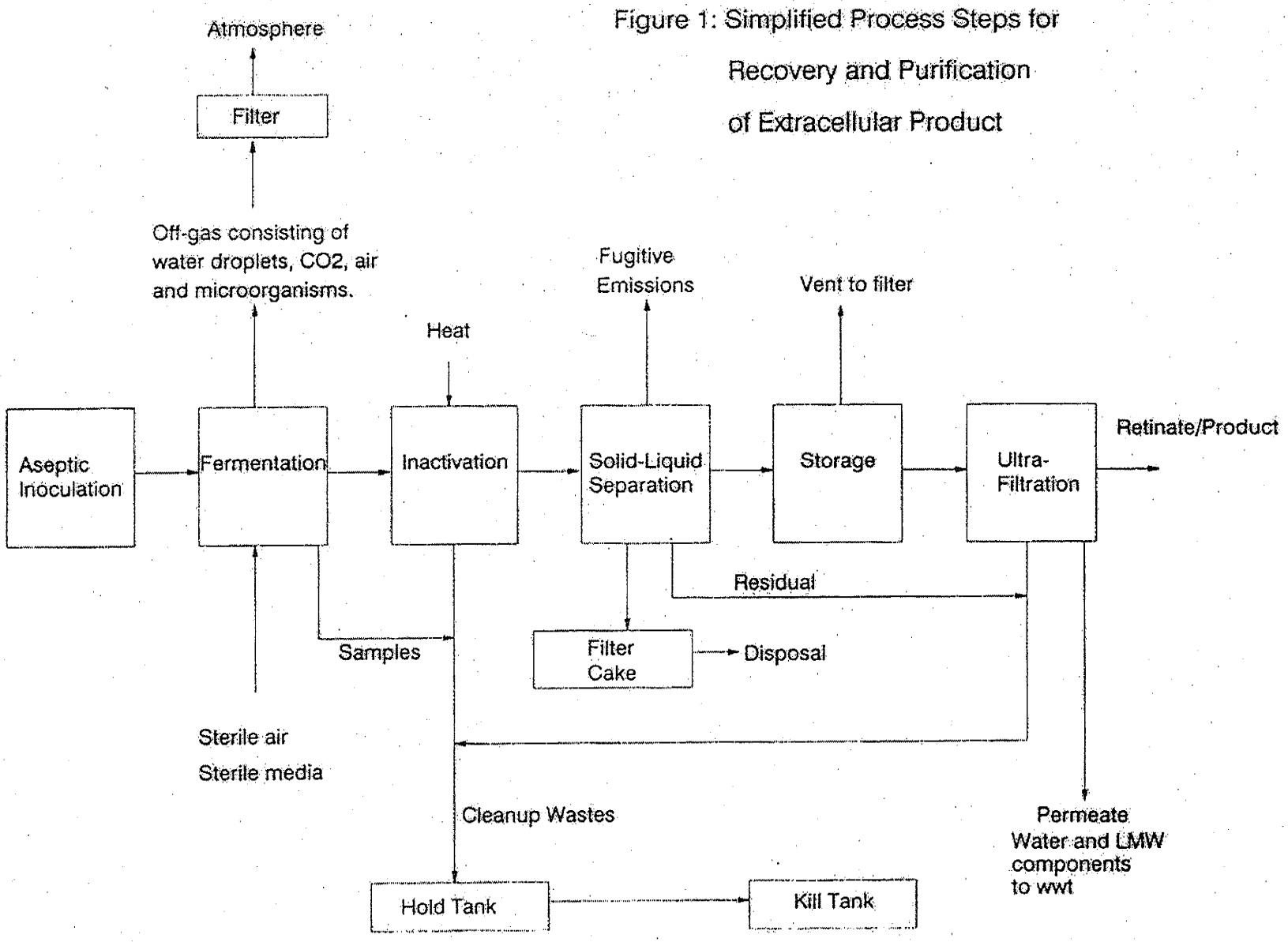
II. Fermentation Process

Description

Figure 1 is a simplified process flow diagram illustrating the steps involved in a typical fermentation process.

Pure stock culture is transferred to a shake flask containing prepared medium. After 10 to 14 hours, the contents of the shake flask are aseptically transferred to a sterilized canister and used to inoculate the seed fermentors. Based on previous assessments of PMN's, the seed fermentors are assumed to have a total culture volume of 3,000 l. The inoculated seed fermentors are operated at slightly elevated temperatures, in the range of 25 to 50 deg C, with moderate aeration and agitation for several hours. After cooling, the contents of the seed fermentor are aseptically

Figure 1: Simplified Process Steps for Recovery and Purification of Extracellular Product



transferred to the production fermentor.

The production fermentor is a closed pressure vessel equipped with an external jacket, a sterile air supply, agitators, and a sparger. The stirred-tank fermentor is the dominant design in the fermentation industry- an estimated 90% of the medium to large-scale processes use stirred-tank fermentors (Radian, 1986). Based on previous assessments of PMN's, the fermentation system is assumed to have a total culture volume of about 70,000 liters/batch. Prior to inoculation, the production fermentor is cleaned with a caustic wash and then steam sterilized while empty. The nutrient medium is prepared, transferred to the production fermentor, and sterilized in situ. The contents of the seed fermentors are transferred to the process fermentor and additional nutrients, chemicals to adjust pH, and anti-foaming agents added as necessary.

During the fermentation, the vessel is continually sparged with oxygen. Variables such as temperature, pH, and dissolved oxygen will be automatically controlled. The process is expected to take up to 4 days/batch.

The next stage of the process is to extract the desired product from the fermentation broth, an aqueous solution consisting of intact microorganisms, cell debris, low concentrations of the desired product, medium components, and other metabolic components. The extract is then purified, concentrated, and stabilized.

Generally, solid particles, microbial cells, and other debris are first removed by centrifugation or filtration. For the purposes of this model, the broth is assumed to be transferred to

the trough of a rotary drum filter. Rotary drum filters are the generally used in the fermentation industry for large volume continuous filtration of liquids with high solids concentrations. As the filter rotates, a vacuum is pulled inside the filter, picking up the slurry. The bulk of the cell mass is collected on the outside of the filter, then scraped off with a knife and deposited on a conveyor belt. The expected efficiency of solids removal for the rotary drum filter is 99%.

An ultrafiltration system is expected to be used to further concentrate the product. Ultrafiltration employs resin membranes with pore sizes in the range of 0.001 to 0.02 micrometers and molecular weight cutoffs of 300 to 300,000 daltons. These membranes allow low molecular weight components to pass through while retaining the high molecular weight components and cell debris. The product leaving the ultrafiltration system is assumed to be free of viable microorganisms.

III. Releases

A. Air

Air emissions are expected from fermentor vents, openings, seals, and fittings, emergency relief valves, sampling operations, the rotary drum filter, and storage tank vents. The two major sources of releases- the fermentor off-gas and fugitive emissions from the rotary drum filter have been quantified.

Fermentor off-gases generally consist of CO_2, air, and entrained water droplets and microorganisms. One unpublished study found the airborne concentration in the off-gases from a lab scale

fermentor to range from $4x10^5$ to $1x10^8$ droplets/m^3 depending on the impeller speed and density of the broth (Pan, 1990). For comparison, earlier studies suggest that bacterial loading in fermentor off-gases range from $5x10^3$ to $5x10^5$ cfu/ft^3, with a reasonable average being $4x10^4$ cfu/ft^3 (equivalent to $1.8x10^7$ cfu/m^3) (Battelle, 1986). In addition, the laboratory study determined that 30 to 40% of the droplets had diameters greater than 2 micrometers (um) (Pan, 1990). Assuming that droplets with diameters of less than 2 um are too small to carry microorganisms and neglecting the die-off rate of the organisms in the aerosol, the airborne emissions of microorganisms from the laboratory fermentor are estimated to range from $7.8x10^2$ to $1.9x10^5$ cfu/m^2/sec. Assuming similar emission rates for a large scale fermentor, uncontrolled air emissions are estimated to range from $2x10^8$ to $1x10^{11}$ cfu/day throughout the year.

For contained systems, treatment of vent gases from the fermentor is expected. In many cases, microorganisms can be contained by installing a filter in the gas line. Filtration efficiency will be dependent on organism loading, organism size, air velocity, and rated pore size of the filter. A 99% filtration efficiency must be achieved under normal operating conditions to meet the criteria for the exemption. Thus, controlled air emissions are expected to range from $2x10^6$ to $1x10^9$ cfu/day throughout the year.

The other major source of air emissions is the rotary drum filter. Based on limited data submitted for PMN's concerning the generation of aerosols from the rotary drum filter, emissions are

expected to be on the order of 250 cfu/day throughout the year for both the uncontrolled and contained system.

B. Water and Solids

Contaminated wastes generally require treatment to ensure that viable organisms or transmittable genetic materials are not released. Thus, an integral part of bioprocesses using recombinant organisms is the inactivation or "cell kill." Cell kill within the fermentor by chemical or thermal treatment is a recommended procedure for many processes. This practice reduces the need for physical containment of the microorganism in the downstream processing. For cases in which the cell kill method may damage the product and reduce yield, the cell harvesting operations must be contained and the cell kill is incorporated into early downstream product recovery steps. The choice of an inactivation method is based on effectiveness and economics. Common methods employ heat or chemicals. Data have been collected to describe the effectiveness of commonly-used methods of inactivation of bacteria, viruses, and fungi.

Sources of aqueous releases include steam condensate streams from the sterilization of the fermentor vessel and filters, disposal of samples, residues in the rotary filter trough, and cleaning wastes from the ultrafilter membrane. These wastes may contain viable organisms and will be sent to a kill tank prior to discharge to either onsite waste water treatment (wwt) or publicly-owned treatment works (POTW). Land releases are expected from the disposal of the filter cake and typically are sent to landfill or

spread onto land. Assuming a maximum cell density for the fermentation broth of 10^{10} microbes/ml for bacteria, a batch volume of 70,000l, and a minimum log reduction of 2 from the inactivation procedures, releases to water are expected to be about $7x10^{13}$ cfu/day and releases to land are expected to be about $7x10^{15}$ cfu/day. To meet the requirements for the full exemption, submitter's will have to demonstrate a 6 log reduction from inactivation procedures. Assuming a 6 log reduction, aqueous wastes are estimated to be $7x10^{9}$ and solid wastes are estimated to be $7x10^{11}$. Releases of fungi have been estimated similarly based on an expected concentration in the broth of 10^{9} microbes/ml. The releases of bacteria and fungi are summarized in Tables 1 and 2.

TABLE 1: SUMMARY OF RELEASES OF GENETICALLY-ENGINEERED BACTERIA FOR TYPICAL FERMENTATION PROCESS

MEDIA	Minimally Controlled[1] (cfu/day)	Contained System[2] Meeting Full-Exemption (cfu/day)	Days/yr
Air			
Vents	$2x10^{8}$ to $1x10^{11}$	$2x10^{6}$ to $1x10^{9}$	350
Rotary Drum Filter	250	250	350
Water (to wwt)	$7x10^{13}$	$7x10^{9}$	90
Solids	$7x10^{15}$	$7x10^{11}$	90

1 Assume no treatment of fermentor off-gas. Assume a 2-log reduction relative to the maximum cell density of the fermentation broth resulting from inactivation.

2 Assume use of in-line filters to treat vent gases and a 99% removal efficiency under normal operating conditions. Assume an overall 6-log reduction relative to the maximum cell density of the fermentation broth resulting from inactivation steps.

TABLE 1: SUMMARY OF RELEASES OF GENETICALLY-ENGINEERED FUNGI FOR TYPICAL FERMENTATION PROCESS

MEDIA	Minimally Controlled[1] (cfu/day)	Contained System[2] Meeting Full-Exemption (cfu/day)	Days/yr
Air			
Vents	$2x10^8$ to $1x10^{11}$	$2x10^6$ to $1x10^9$	350
Rotary Drum Filter	250	250	350
Water (to wwt)	$7x10^{12}$	$7x10^8$	90
Solids	$7x10^{14}$	$7x10^{10}$	90

1 Assume no treatment of fermentor off-gas. Assume a 2-log reduction relative to the maximum cell density of the fermentation broth resulting from inactivation.

2 Assume use of in-line filters to treat vent gases and a 99% removal efficiency under normal operating conditions. Assume an overall 6-log reduction relative to the maximum cell density of the fermentation broth resulting from inactivation steps.

C. Obligate Anaerobes

Several of the microorganisms which are being evaluated for hazard are obligate anaerobes (can not function in the presence of oxygen). With the exception of the production of some alcohols and the digestion of organic wastes, no commercial bioprocesses utilizing anaerobes have been identified. Commercial anaerobic fermentation processes are currently at the research stage. Releases from an anaerobic process have not been quantified but are expected to be no greater than those estimated for the aerated fermentor.

D. Spores

Several of the microorganisms being considered for exemption are spore-formers. The PMN's submitted to date have involved sporulation deficient cells. Assuming a sporulation rate of 1 in 10 million cells, a cell density of 10^{10} cfu/ml and a batch volume of 70,000 l, an estimated $7x10^{10}$ spores/batch may be formed. Bacterial spores are highly resistant to destruction. Based on information submitted in previous PMN's, inactivation methods which are highly effective against vegetative cells may have no effect on spores. Assuming that the inactivation procedure is ineffective against the spores and a separation efficiency for the rotary drum filter of 99%, worst case estimates of the releases are $1.7x10^{10}$ spores/day in solid wastes and less than $2x10^{8}$ spores/day in aqueous wastes. These estimates are in addition to the release estimates presented in Table 1.

IV. Occupational Exposures

Occupational exposures may potentially occur during activities such as pipetting in the lab, sampling, inoculation, and changing air filters. A typical site will employ less than 10 workers/shift and operate 24 hours/day throughout the year.

NIOSH has conducted walkthrough surveys of several fermentation facilities in the enzyme industry and monitored for microbial air contamination. These facilities were not using rDNA microorganisms but the processes were considered fairly typical of fermentation process technology. Area samples were taken in locations where the potential for worker exposure was considered to be potentially greatest- near the fermentor, the seed fermentor, sampling ports, and separation processes (either filter press or rotary drum filter). The workers with the highest potential average exposures at the three facilities visited were those involved in sampling. Area samples near the sampling port revealed average airborne concentrations ranging from 350 to 648 cfu/m^3. Typically, the Chemical Engineering Branch would not use area monitoring data to estimate occupational exposure levels since the correlation between area concentrations and worker exposure is highly uncertain. Personal sampling data is not available at the present time. Thus, area sampling data has been the only means of assess exposures for previous PMN biotechnology submissions. Assuming that 20 samples per day are drawn and that each sample takes up to 5 minutes to collect, the duration of exposure for a single worker will be about 1.5 hours/day. Assuming that the

concentration of microorganisms in the worker's breathing zone is equivalent to the levels found in the area sampling, the worst case daily inhalation exposure is estimated to range up to 650 to 1200 cfu/day. The uncertainty associated with this estimated exposure value is not known.

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